THE DISTRIBUTION OF FUNGI IN THE HORIZONS OF A PODSOLISED SOIL

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Introduction

This study was performed on an iron-humus podsol at Delamere forest, Cheshire where the tree cover consisted predominantly of a stand of *Pinus sylvestris*. Such soils, with their clearly defined horizons and acid reaction, have received a good deal of attention from soil mycologists.
BISBY, TIMONIN and JAMES (1935) conducted extensive surveys of podsolised soils in Canada, relating the distribution of fungi to depth, season and other factors. In 1951, WARCUP studied the vertical distribution of fungi in several soil types and his approach has since been adopted by other workers. In Australia, MCLENNAN and DUCKER (1953, 1954) investigated populations of acid sandy podsols. SEWELL (1959) studied fungal ecology in a range of British podsols and a similar study was made by BROWN (1958) on heath podsols associated with various sand dune systems. Most of these studies were performed using the soil plate or dilution plate techniques; these methods are known to be selective for isolates originating from spores (WARCUP, 1955, 1960). The investigation reported here was undertaken using a soil washing technique (PARKINSON and WILLIAMS, 1961) which was designed to separate microhabitats from soil while removing most spores from them. Thus it was hoped to study fungi present in a vegetative state in soil. This work was conducted as a supplement to studies of soil respiration and soil sections of the same soil.

Method

The soil washing was performed using perspex boxes fitted with 3 stainless steel sieves of graded size. A soil sample was serially washed under aseptic

conditions by passage of sterile compressed air through the boxes. The resulting agitation gradually broke up aggregates and released spores held by them. On completion of washing, discrete soil particles are distributed on the sieves according to their size. When such particles were plated, chances of obtaining isolates from hyphae were considerably greater than when unwashed soil was plated. The spore content of a soil sample could also be assessed by plating collected drainage water after washing. Thus a more complete assessment of the fungus content could be obtained.

The sampling procedure employed was as follows. A pit (1 yd. x 1 yd.) was dug to expose all horizons and 10 samples were taken from each horizon. In the laboratory, the samples of each horizon were bulked and thoroughly mixed; from these bulked samples, weighed sub-samples were taken and placed on the top sieve of a washing box. The boxes were then sealed and the 5 soil samples subjected to a pre-determined number of serial washings:

A1 : 30; A2 : 20; B1 : 30; B2 : 30; C : 10;

On completion of washing, discrete soil particles were picked out and plated onto pre-poured plates of Czapek-Dox medium with 0.65 % yeast extract and adjusted to pH 5.0. Various types of particle were easily recognised and were plated separately (root fragments, humus particles, and mineral grains of various size). All drained washing water from each horizon was collected and used to prepare dilution plates.

Results and Discussion

As can be seen from tables 1 and 2, more species were obtained from washed soil than from washing water dilution plates. All species isolated from washing water also appeared on washed particles and 21 types obtained from washed soil were absent from washing water. These included many sterile forms, together with a few Basidiomycetes and Ascomycetes.

The dominant types in each horizon, as assessed by both methods, are shown in table 3, In the A1 horizon, *Mortierella parvispora*, *Penicillium spinulosum*, and *Trichoderma viride*, were dominant on washed soil, with *Gliomastix* sp. and *Chrysosporium* sp. also prevalent in washing water. In the A2 horizon, the same three species were dominant in washed soil and washing water. In the B1, B2, and C horizons, *Phialophora* sp. predominated on washed soil but not in washing water; this fungus remained sterile under normal cultural conditions, fruiting being induced when it

TABLE 1. Species isolated from washed soil

	A1	A2	B1	B2	C
Ascomycetes					
* *Coniochaeta* sp.		x			
* *Fraseriella* sp.	xx	x	x		
* *Perisporium vulgare* CORDA			x		
Basidiomycetes					
* S.C. 1					x
* S.C. 2				x	
* S.C. 3				x	x
* S.C. 4				x	
Phycomycetes					
* *Mortierella isabellina* ZYCHA	x	x			
,, *macrocystis* GAMS	x		x		
,, *parvispora* LINNEMANN	xxxx	xx	xx	x	x
Mucor ramannianus MOELLER	xx	xxx	xxxx	xx	xx
,, ,, (forma *intermedia*)		x	x		
* *Mucor hiemalis* WEHMER	x	x			
Fungi imperfecti					
Aspergillus sp.	x		x		
Cephalosporium sp.	x		x		
Chrysosporium sp.	xx	xx	xx	x	
Gliomastix sp.	xx	xx	xx	x	x
Humicola sp.	xx	x	x		
Oidiodendron fuscum ROBAK	x	x	x	xx	xx
Paecilomyces sp.			x	xxx	x
Penicillium lanosum WESTLING	x	x			
,, *spinulosum* THOM	xxx	xx	xx		
,, sp. (1)	x	x	x		
,, sp. (2)	x				
,, sp. (3)	xx	x	x		
Phialophora sp.	xx	xx	xx	xxx	xx
Sporotrichum sp.	x	x			
Trichoderma album PREUSS	xx	xx	xx	xx	x
,, *viride* PERS	xxx	xx	xx	x	
Sterile isolates					
Hyaline S.H. 1	xx	xx	xx		
* S.H. 2	x	xx	x	x	
S.H. 3	x	x	x		
* S.H. 4		x	x		
* S.H. 5		x			
* S.H. 6		x			
* S.H. 7		x			
* S.H. 8	x				

TABLE 1 (continued)

		A1	A2	B1	B2	C
*	S.H. 9			x		
*	S.H. 10				x	
* Dark	S.D. 1			x	x	
*	S.D. 2	x		x	x	
*	S.D. 3				x	x
*	S.D. 4			x		

* = Species not isolated from washing water,

% particles colonised
x = 1 %
xx = 1—10 %
xxx = 11—20 %
xxxx = 21—30 %

TABLE 2. Species isolated from washing water

	A1	A2	B1	B2	C
Phycomycetes					
Mortierella macrocystis GAMS	x	x	x		
,, *parvispora* LINNEMANN	xxxx	xx	xxx	x	x
Mucor ramannianus MOELLER	x	xx	xxx	xx	x
,, ,, (forma *intermedia*)		x	x		
Fungi imperfecti					
Aspergillus sp.	x		x		
Cephalosporium sp.	x				
Chrysosporium sp.	xxx	xx	xxx	x	
Gliomastix sp.	xxx	xx	xx	x	
Humicola sp.	xx	x	x		
Oidiodendron fuscum ROBAK	xx	x	x	x	x
Paecilomyces sp.			x	xx	x
Penicillium lanosum WESTLING	xx	x	x		
,, *spinulosum* THOM	xxx	xx	xx	x	
,, sp. (1)	x		x		
,, sp. (2)			x		
,, sp. (3)	xx	x	x	x	
Phialophora sp.	x	x	x	x	x
Sporotrichum sp.	x	x			
Trichoderma album PREUSS		x	x	x	x
,, *viride* PERS.	xx	x	x		

TABLE 2. (continued)

	A1	A2	B1	B2	C
Sterile isolates					
Hyaline S. H. 1		x	x		
S.H. 3	xx	x			

Cols. per g dry wt. soil

x = 1,000
xx = 1,000—10,000
xxx = 10,000—20,000
xxxx = 20,000

TABLE 3. The dominant fungi in each horizon of the Delamere forest soil

	Washed soil	% Parts. colonised	Washing water	No. cols. per g dry wt. of soil
A1	*Mortierella parvispora*	22.9	*Mortierella parvispora*	51,500
	Penicillium spinulosum	12.5	*Gliomastix* sp.	15,400
	Trichoderma viride	11.9	*Chrysosporium* sp.	12,700
			Penicillium spinulosum	12,700
A2	*Mucor ramannianus*	12.0	*Mucor ramannianus*	8,000
	Mortierella parvispora	7.5	*Mortierella parvispora*	7,400
	Chrysosporium sp.	6.0	*Chrysosporium* sp.	4,700
B1	*Mucor ramannianus*	24.9	*Mucor ramannianus*	15,300
	Phialophora sp.	9.4	*Mortierella parvispora*	11,100
	Mortierella parvispora	6.6	*Chrysosporium* sp.	10,800
B2	*Phialophora* sp.	15.3	*Paecilomyces* sp.	3,900
	Paecilomyces sp.	10.8	*Mucor ramannianus*	1,900
	Mucor ramannianus	7.4	*Oidiodendron fuscum*	900
C	*Phialophora* sp.	4.9	*Paecilomyces* sp.	100
	Mucor ramannianus	1.3	*Mucor ramannianus*	100
	Oidiodendron fuscum	1.1	*Mortierella parvispora*	30

was grown on carboxymethyl-cellulose medium (GAMS, 1960) at 2°C for 2 months. It is clear from these results that the Phycomycetes *M. parvispora* and *M. ramannianus* played an important role in all horizons.

TABLE 4. The distribution of the dominant fungi through the profile

Fungi which decrease with increasing depth	Fungi which have maxima at lower depths
Mortierella parvispora	*Mucor ramannianus*
Penicillium spinulosum	*Phialophora* sp.
Trichoderma viride	*Trichoderma album*
Humicola sp.	*Paecilomyces* sp.
Gliomastix sp.	*Oidiodendron fuscum*
Chrysosporium sp.	

On the basis of their vertical distribution through the profile, fungi could be placed in one of two groups (table 4). Most species predominating in the A1 horizon decreased in frequency with increasing depth, being absent or in low frequency in the B2 and C horizons. Other types had maxima in one of the lower horizons, thus comprising the sub-surface microflora of this soil. Generally the distribution of fungi was not sharply affected by change of horizon. An exception to this was *Paecilomyces* sp. which had a clear maximum in the B2, low frequency in the C and B1, and was absent from the A1 and A2 horizons.

There were no qualitative differences between fungi isolated from mineral and organic particles. This would be anticipated as most fungi developing from mineral grains originated from coatings of organic matter. Most species were isolated with decreased frequency from mineral particles and the overall colonisation of the grains was much reduced compared with that of organic particles. Colonisation of the mineral grains generally decreased with the size of the particles.

The dominant colonisers of root fragments and humus particles are shown in table 5. Generally the same fungi were dominant on both substrates, depth appearing to be a more important selective factor than substrate. However one species, *Phialophora* sp. showed a clear preference for root fragments. This species has also been isolated from the inner cortex of live roots of *Pinus sylvestris* and from *Pinus nigra* roots. It is probable that most of the dead root fragments isolated from the Delamere soil originated from lateral roots of *Pinus sylvestris*.

TABLE 5. The dominant fungi on root fragments and humus particles in each horizon (Recorded as % of particles colonised)

	Root fragments		Humus particles	
A1	*Mortierella parvispora*	44.6	*Mortierella parvispora*	35.8
	Penicillium spinulosum	25.4	*Trichoderma viride*	20.0
	Trichoderma viride	23.0	*Penicillium spinulosum*	18.0
A2	*Phialophora* sp.	14.1	*Mucor ramannianus*	23.8
	Mucor ramannianus	13.9	*Mortierella parvispora*	17.3
	Gliomastix sp.	12.6	*Chrysosporium* sp.	11.8
B1	*Mucor ramannianus*	38.8	*Mucor ramannianus*	57.3
	Phialophora sp.	34.9	*Chrysosporium* sp.	12.0
	Mortierella parvispora	13.9	*Mortierella parvispora*	11.3
B2	*Phialophora* sp.	49.5	*Mucor ramannianus*	19.6
	Mucor ramannianus	12.9	*Paecilomyces* sp.	19.1
	Trichoderma album	6.0	*Phialophora* sp.	14.6
C	*Phialophora* sp.	21.0	*Oidiodendron fuscum*	5.3
	Trichoderma album	5.6	*Phialophora* sp.	4.8
	Mucor ramannianus	2.5	*Trichoderma album*	3.1

Consideration of data from each of 8 samplings taken throughout the year did not reveal any significant quantitative or qualitative seasonal fluctuations in fungal populations. There was some evidence that soil moisture was an important factor controlling fungal activity (see also PARKINSON and COUPS, 1962) On the system of sampling used, variations in seasonal samples could be duplicated when samples from laterally spaced sites were taken at one time.

The results of this floristic study of an acid podsolised soil bare many similarities to those obtained by other workers studying similar soils. (BISBY, TIMONIN and JAMES, 1935; JEFFREYS et al., 1953; MCLENNAN and DUCKER, 1954; CHESTERS and THORNTON, 1956; BROWN, 1958; SEWELL, 1959). However several species, which do not normally appear in lists of isolates from such soils, were detected by use of the soil washing technique. (*Phialophora* sp., Basidiomycetes, Ascomycetes and sterile forms). Also the method allowed study of the fungal colonisation of various microhabitats in the soil.

Summary

A soil washing technique was applied to a study of fungi in the horizons of an iron-humus podsol at Delamere forest, Cheshire. An attempt was made to make a distinction between fungi present in a vegetative state and those existing as spores in the soil; the vertical distribution of fungi on various substrates was studied.

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Discussion

G. W. HARMSEN: Was the number of species isolated from washed soil higher than that obtained from unwashed material?

S. T. WILLIAMS: There was a 100 % increase in species isolated from washed soil. Otherwise slow growing forms would be overgrown by the more rapidly developing ones.

G. J. F. PUGH: It is interesting to see that Mr. Williams' most common species are also very active on chitin strips as described by Gray in the H-layer.

W. GAMS: From my work in the H-layer of the Delamere Forest podzol I am rather convinced that these chitin decomposing species of *Penicillium* and *Mortierella* are active in this layer.

D. PARKINSON: The nature (not the amount) of the organic material in the H and A_1 horizons being similar, this might explain the occurrence of *Mortierella parvispora*, *Trichoderma viride* and *Penicillium* spp. in both these horizons.

W. LOUB: Can you find fast growing fungi on your washed particles throughout the year?

S. T. WILLIAMS: Yes, independent of seasons.

B. N. K. DAVIS: Can you tell me the extent to which particle size does affect the microflora? Did you find different species on different sized particles?

S. T. WILLIAMS: There was a quantitative decrease with particle size but no species difference.